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Structure-activity relationships for insect kinins on expressed receptors from a tick (Acari: Ixodidae) and a mosquito (Diptera: Culicidae) *)

Suparna TANEJA-BAGESHWAR[1], Allison STREY[2], Pawel ZUBRZAK[2], Patricia V. PIETRANTONIO[1*] and Ronald J. NACHMAN[2**]

*[1] Department of Entomology, Texas A&M University, College Station TX 77843-2475, USA, *e-mail: p-pietrantonio@tamu.edu*
[2]Areawide Pest Management Research, Southern Plains Agricultural Research Center, ARS, USDA, 2881 F/B Road, College Station, TX 77845, USA
***e-mail: nachman@tamu.edu*

Abstract: The systematic analysis of structure-activity relationships of insect kinins on two heterologous receptor-expressing systems is described. Previously, kinin receptors from the southern cattle tick, *Boophilus microplus* (Canestrini, 1888) [1, 2] and the dengue vector, the mosquito *Aedes aegypti* (L.) [3] were functionally and stably expressed in CHO-K1 cells. In order to determine critical kinin residues for the peptide-receptor interaction, kinin core analogs were synthesized as an Ala-replacement series of the peptide FFSWGa and tested by a calcium bioluminescence plate assay. The amino acids Phe[1] and Trp[4] were essential for activity of the insect kinins in both receptors. It was confirmed that the pentapeptide kinin core is the minimum sequence required for activity and that the C-terminal amide is also essential. The aminoisobutyric acid (Aib)-containing analog, FF[Aib]WGa, was as active as superagonist FFFSWGa on the mosquito receptor in contrast to the tick receptor where it was statistically more active than FFFSWGa by an order of magnitude. This restricted conformation Aib analog provides information on the conformation associated with interaction of the insect kinins with these two receptors. This analog is an important lead in the development of biostable insect kinin analogs for blood-feeders control.

Keywords: kinin receptor, Ala replacement series, calcium bioluminescence plate assay, insect G protein-coupled receptor

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INTRODUCTION

Most leucokinins are characterized by the C-terminal pentapeptide Phe-Xaa-Ser-Trp-Gly-NH_2 where X is Phe, His, Ser or Tyr [4]. Myotropic and diuretic assays of tissues *in vitro* show that the activity of the insect kinins resides in the C-terminal pentapeptide, the active core [4, 5]. Diuretic and myotropic activity was lost when the C-terminal amide of the insect kinins is replaced with a negatively charged acid moiety [6]. Within the pentapeptide, residues Phe^1 and Trp^4 are the most important for activity [4, 7]. One receptor interaction model proposes that the aromatic side chains of Phe^1 and Trp^4 are oriented towards the same region and interact with the receptor. Conversely, the side chain of residue 2 lies on the opposite face pointing away from the receptor surface, which explains why this position is more tolerant to changes [8]. Myokinin receptors from the southern cattle tick, *Boophilus microplus* [1, 2] and the dengue vector, the mosquito *Aedes aegypti* [3] were previously functionally expressed in CHO-K1 cells, in which they elevate intracellular calcium in response to kinins/ kinin analogs [1, 3]. Here we present an analysis of structure-activity relationships of C-terminal pentapeptide core of the insect kinins on these two receptor-expressing systems. In order to determine which myokinin residues are critical for the peptide-receptor interaction, kinin core analogs were synthesized as an Ala-replacement series and were tested by a calcium bioluminescence assay [3]. We also determined the minimal size of an active myokinin core and the effect of the C-terminal OH group on the myokinin receptor response. We evaluated a restricted conformation analog of the insect kinins, previously shown to have potent activity in a diuretic assay [9], that incorporates the sterically-bulky residue aminoisobutyric acid (Aib).

MATERIALS AND METHODS

Peptide analogs, Cell lines and analysis of myokinin peptide analogs

The analogs synthesis, HPLC and amino acid analysis conditions were as described [10]. The CHO-K1 cell lines expressing the tick receptor (BmLK3) and the *Aedes* kinin receptor (E10) were maintained as described [1-3]. The functional analysis of peptides on these cell lines was by a Ca^{2+} bioluminescence assay [3].

RESULTS

Effect of Ala substitution on the activity of the insect kinin core

The kinin core, FFSWGa, and five different Ala substituted analogs of this peptide were synthesized and tested on tick and mosquito kinin receptors expressed in CHO-K1 cells using a calcium bioluminescence assay. The analogs were studied at concentrations from 10 μM to 10 nM. On the tick receptor (BmLK3 cell line), the analog FF**A**WGa elicited the greatest response at 1 μM followed by FFSW**A**a and FFSWGa (equal response), and lastly, with apparent lesser activity, F**A**SWGa. Analogs **A**FSWGa and FFS**A**Ga failed to show any response on the tick receptor. On the mosquito receptor (E10 cell line) the most potent analog was also FF**A**WGa followed in potency by FFSW**A**a as observed for the tick receptor. Analogs FFS**A**Ga and **A**FSWGa did not show any response on the mosquito receptor. Contrary to the response on the tick receptor, analogs F**A**SWGa and FFSWGa showed little response even at 1μM on the mosquito receptor. The analog FFSWGa was statistically less potent than FFFSWGa on the tick receptor.

Minimal size and terminal OH group

Two analogs, FFSWa and FSWGa, were designed to confirm the minimal size required for activity and one analog having the OH group at its C terminus, FFSWG-**OH,** was designed to determine the role of the C-terminal amide. All analogs failed to elicit responses on both receptors even at concentrations of 10 μM, as expected, verifying that the minimum fragment required for activity is a pentapeptide with a C-terminal amide.

Restricted conformation analog

The Aib-containing analog, FF[Aib]WGa, exhibited a response comparable to that of the hexapeptide FFFSWGa at 1 μM concentration, both on the tick and mosquito receptors. The hexapeptide had been previously found to be most potent among different peptides in activating the tick myokinin receptor in a fluorescence calcium assay with the same cell line [1]. In this study the hexapeptide elicited the greatest response at 1 μM for both tick and mosquito receptors. The Aib analog was more potent on the tick receptor than FFFSWGa. In contrast, both peptides were equipotent on the mosquito receptor.

DISCUSSION

The pentapeptide analog truncations at the C- and N-terminus and the C-terminal amide replacement resulted in loss of activity on both receptors. This demonstrates that the C-terminal pentapeptide represents the minimal core required to elicit a receptor response. The analog FFSWG-**OH** failed to elicit a response in either case. As observed with diuretic and myotropic assays [4, 11, 12], the C-terminal amide is critical for interaction of the insect kinins in these receptor systems. Evaluation of a series of Ala-substituted analogs of the C-terminal pentapeptide FFSWGa demonstrates that two of the analogs, **A**FSWGa and FFS**A**Ga, were completely inactive on both tick and mosquito receptors. This demonstrates the requirement of the aromatic side chains of Phe^1 and Trp^4 for the activity of the insect kinin core, noted in earlier studies with diuretic and myotropic assays [4, 8, 13]. The mosquito receptor, however, responds strongly to the addition of a Phe at the N-terminus of the kinin core. The hexapeptide FFFSWGa elicits a significantly stronger response at I μM than any of the other active pentapeptide analogs. Despite the steric bulk in the backbone of the Aib-containing analog FF[Aib]WGa, it nevertheless elicits a strong response in both tick and mosquito receptors. This is in agreement with the potent activities of Aib containing analogs observed in a cricket Malphigian tubule fluid secretion assay, *in vivo* housefly diuretic assay and a cockroach hindgut myotropic assay [9, 14]. The steric bulk of the Aib residue also restricts the number of conformations available to the backbone of this analog, and provides insight into the conformation adopted by the insect kinins at the two receptors. In conclusion, the potent activity of FF[Aib]WGa is an interesting observation given that the steric bulk of the Aib residue confers resistance to degradation by peptidases such as ACE and nephrilysin that attack the native insect kinins at the peptide bond between the Ser^3 and Trp^4 core residues [14]. This can be an important lead in the development of biostable insect kinin analogs that cannot be deactivated by ticks and mosquitoes.

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